Population Genetic Analysis of a Global Collection of *Pyrenophora tritici-repentis*, Causal Agent of Tan Spot of Wheat

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ABSTRACT

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The work presented here is the first major study to analyze the genetic diversity within the worldwide population of the economically important wheat pathogen *Pyrenophora tritici-repentis*. The genetic structure of field populations of *P. tritici-repentis* was determined using amplified fragment length polymorphism markers along with sequence data from the internal transcribed spacer region of the ribosomal DNA. Ninety-seven fungal isolates were collected from naturally infected wheat and wild grass species. The collection of 97 *P. tritici-repentis* isolates included races 1, 2, 3, 4, 5, ND7, and ND8 and was collected from North America, South America, and Europe. Results show no genetic grouping of fungal races nor do results indicate grouping based on geographic location indicating that the population is preferentially outcrossing in nature and that the introduction and spread of this population is either relatively recent or that there has been a constant worldwide flow of this fungus possibly by seed movement between continents.

Additional keywords: ascomycete, *Drechslera tritici-repentis*.

Pyrenophora tritici-repentis (anamorph, *Drechslera tritici-repentis*), causal agent of tan spot of wheat, is an economically important pathogen in many wheat growing regions of the world. The fungus causing tan spot was first described in 1823 (17) and has since been found on all continents and in most major wheat growing regions of the world. This fungus was identified on grasses in Germany in 1902 (11) and described on *Agropyron repens* in the United States in 1923 (12) and on wheat in Japan in 1928 (28). After 1928 the fungus was reported as a saprophyte or a cause of minor spotting or occasionally as a cause of severe spotting in wheat growing regions throughout the world (17). In 1963, pink smudge of wheat seed was shown to be associated with the fungus causing yellow leaf spot (tan spot) of wheat (36) showing the potential for seed transmission of this fungus. In 1968 and 1969, tan spot was reported as severely pathogenic on wheat in North Dakota (16).

Hosford (16) proposed a disease cycle and a rating scale for disease and showed that wheat lines varied in resistance and isolates of the fungus varied in pathogenicity. Since this time, researchers have attempted to elucidate the mechanisms of the tan spot host–pathogen interactions. Lamari and Bernier (20) identified four pathotypes of *P. tritici-repentis* based on necrotic and chlorotic lesion production on wheat. Pathotype 1 produces both necrosis and chlorosis (nec+chl+), pathotype 2 produces necrosis only (nec+chl–), and pathotype 3 produces chlorosis only (nec–chl+). Pathotype 4 produces neither necrosis nor chlorosis (nec–chl–) and is nonpathogenic on wheat.

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In 1995, Lamari et al. (21) described a second type of chlorosis that was identified based on a differential reaction where chlorosis was induced on wheat cv. Katepwa but not on cv. 6B365. This led them to propose a race classification system for *P. tritici-repentis* isolates. Isolates that produced necrosis on the differential cv. Glenlea and chlorosis on the differential line 6B365 but did not produce chlorosis on the differential cv. Katepwa were designated as race 1. Isolates that produced necrosis on Glenlea but did not produce chlorosis on 6B365 or Katepwa were designated as race 2. Isolates that produced chlorosis on 6B365 but not Katepwa and did not produce necrosis on Glenlea were designated as race 3. Race 4 isolates are avirulent on wheat, and isolates designated as race 5 produced chlorosis on Katepwa but not on 6B365 and did not produce necrosis on Glenlea. Lamari et al. (22) documented the finding of the remaining combinations of symptoms that filled out a total of eight races. Race 6 combines virulence of races 3 and 5. Race 7 combines virulence of races 2 and 5, and race 8 combines virulence of races 2, 3, and 5.

Five toxins of *P. tritici-repentis* Ptr ToxA (33), Ptr ToxB (29), Ptr ToxC (13), and two different toxins simultaneously named Ptr ToxD (24,26) have been reported. Ptr ToxA (5,6,34,35,42) and Ptr ToxB (25,32) have been the most well characterized. According to the proposed race classification system (22), Ptr ToxA is produced by races 1, 2, 7, and 8; Ptr ToxB is produced by races 5, 6, 7, and 8; and Ptr ToxC is produced by races 1, 3, 6, and 8. The tan spot host–pathogen system has been the subject of recent reviews (7,10,31).

P. tritici-repentis is a homothallic fungus that readily produces the sexual stage on field stubble giving the fungal population the opportunity for adaptation by sexual recombination. Previous to this study, little information was available on the genetic variation in the fungal population at the molecular level, especially between virulent and avirulent isolates. Therefore it was our goal to identify the extent of *P. tritici-repentis* population diversity. In this study, amplified fragment length polymorphisms (AFLPs) and

ribosomal internal transcribed spacer (ITS) sequence analyses were used to evaluate 97 *P. tritici-repentis* isolates representative of races 1, 2, 3, 4, 5 (22), ND7 (26), and ND8 (2) and one isolate each from the closely related species *P. teres* and *P. bromi*. Isolates were recovered from wheat and non-cereal grasses and were collected from Europe, North America, and South America.

Lichter et al. (23) compared the electrophoretic karyotype of a limited number of pathogenic (race 1) and nonpathogenic (race 4) isolates. The chromosome containing *ToxA*, the gene coding for the production of Ptr ToxA (8), was most closely investigated. Although ToxA was absent from the nonpathogenic isolates investigated, other genomic regions of that chromosome in the pathogenic isolate were present on a single smaller chromosome in the nonpathogenic isolate and molecular markers (probes) for these genomic regions were not polymorphic. This led to the conclusion that there were substantial differences in chromosome composition between pathogenic and nonpathogenic isolates but that lack of molecular marker polymorphism indicated that the isolates could be closely related.

In the present study, our goal was to use a worldwide population of *P. tritici-repentis* to evaluate the level of genetic diversity within the natural population. This collection includes various races both pathogenic and nonpathogenic from different wheat growing areas of the world including regions of North America, South America, and Europe.

MATERIALS AND METHODS

***P. tritici-repentis* isolate collection.** Ninety-seven isolates of *P. tritici-repentis* that had previously been race characterized (1, 2, 3, and 4) or were characterized for this study as described (1) along with control isolates of *P. bromi* and *P. teres* were evaluated. Collections made from 1969 to 2002 were obtained from North America, South America, and Europe (Table 1). Races 1, 2, 3, 4, 5 (21), ND7 (26), and ND8 (2) were used in this study. It should be pointed out that races 1, 2, 3, 4, and 5 are similar in reaction to those described by Lamari et al. (21), whereas races ND7 (26) and ND8 (2) were published simultaneously with races 7 and 8 described by Lamari et al. (22) and therefore do not have the same phenotype on the differential set. All isolates used in this study were single-spored at least twice to ensure genetic purity.

DNA isolation and polymerase chain reaction. For DNA extractions, 250-ml Erlenmeyer flasks containing 100 ml of potato dextrose broth was inoculated with spores of each isolate and incubated at room temperature for 72 h in a rotary shaker at 100 rpm. Mycelium was filtered through Miracloth (Calbiochem Corporation, La Jolla, CA) and washed with sterile distilled water, harvested, and stored at –20°C until ground. Mycelium was ground to a fine powder with liquid nitrogen and a mortar and pestle. Extraction of genomic DNA was done as described (3). ITS regions were amplified by polymerase chain reaction (PCR) using the ITS1 and ITS4 primers (38). Individual reactions included 200 μM each dNTPs, 75 ng each forward and reverse primers, 1 unit of *Pfu* proofreading polymerase (Stratagene, La Jolla, CA), and 40 ng of genomic DNA in 1× *Pfu* polymerase reaction buffer. All PCR was performed on a Dyad Thermal Cycler (MJ Research, Watertown, MA). The cycling parameters were 40 cycles of the following: denaturation, 60 s at 94°C; annealing, 60 s at 55°C; and extension, 3 min at 72°C. PCR was visualized on a 1% agarose gel in a 1× Tris-borate-EDTA (TBE) running buffer. Two PCR reactions were performed for each isolate.

AFLP analysis. AFLP reactions were performed as described (37). All PCR was performed on a Dyad Thermal Cycler (MJ Research). Genomic DNA of each isolate was digested with *Eco*RI and *Mse*I in 1× ligation buffer and ligated to AFLP adaptors (37) in one step for 4 h at 37°C. The digested and ligated templates were diluted 1:10 before being used in the preamplification. Samples were preamplified using the following parameters: 20 cycles of 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s. Following preamplification, the template was diluted 1:40 to be used in selective amplification. Selective amplification was completed using IR700 (LI-COR, Lincoln, NE) labeled *Eco*RI primers with a two base extension (either AG or AC) and an unlabeled *Mse*I primer also with a two base extension (CA). Cycling parameters included one cycle of 94°C for 30 s, 65°C for 60 s, and 72°C for 30 s; this cycle was followed by 12 cycles in which the annealing temperature was lowered each cycle by 0.7°C from 65 to 56°C. This was followed by 23 cycles of 94°C for 30 s, and 56°C for 30 s, and then held at 4°C indefinitely.

AFLP fragments were separated on a LI-COR Global Edition IR^2 System (LI-COR) in a 6% polyacrylamide gel (25 cm in length and 0.25 mm in thickness) made with 1× TBE buffer (LI-COR). This 1× TBE was also used as the running buffer. One microliter of the AFLP reaction mixture was loaded into each well. The LI-COR 700-bp molecular weight standard (LI-COR) was loaded at the left and right lane of each gel. Electrophoresis was done on the Global IR^2 system (LI-COR) using IR700 labeled *Eco*RI primers. Parameters include voltage set at 2,000 V, current at 40 mA, power at 50 W, and temperature at 45°C. Each gel was run for 2.5 h. AFLP fragments were scored by hand and converted into numerical data (presence = 1, absence = 0). These coded data were combined over all AFLP bands within strains and used to define haplotypes. The coded haplotypes of these strains were then entered into the software PopGene (39) for analysis of linkage disequilibria, allele frequency, effective allele number, and genetic diversity index (Table 2). Only bands that gave an effective allele number of 1.20 or greater were used in the calculation of linkage disequilibria, due to the low sample size and consequent lack of statistical power.

DNA sequencing and sequence analysis. Amplified products were gel purified using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA) followed by commercial sequencing using both forward and reverse primers (ITS1 and ITS4) for each of two independent PCRs. A single consensus sequence was generated from alignments of at least two reads for each of two PCRs for each isolate. If a consensus sequence was not obvious from the four sequence alignments, the PCR and sequencing was repeated.

Analysis of the ITS consensus sequences was done using ClustalW sequence analysis software (15), using the consensus sequences derived above coded into FASTA format (30). Output from ClustalX was coded into PHYLIP format (14) for phylogenetic analysis. Sequences derived in this study were compared with sequences in GenBank as follows: AF081446, AF071347, AF400889, AF400890, AF400891, AF400892, AF400893, AF400894, AF400895, AF400896, and AF163061 from *P. teres*; AF071348, AY004807, and AY004809 from *P. bromi*; and AF071348 and AY004808 from *P. tritici-repentis*.

RESULTS

The 99 strains displayed 61 AFLP haplotypes (Table 1). Of these, two belonged to species other than *P. tritici-repentis* (*P. bromi* and *P. teres*) and were not used in the calculations of allelic frequency, number of effective alleles, or linkage disequilibria. The *P. bromi* and *P. teres* strains produced AFLP banding patterns with a high degree of similarity to those of *P. tritici-repentis*. One strain in particular, *P. teres* ND89-19, shares all but 5 of the 55 scored bands with *P. tritici-repentis* strain Ptr 2000-5. Of the 59 remaining haplotypes, 13 were found in more than one strain. Seventeen strains shared one haplotype, nine strains a second, four strains a third, and three shared a fourth, and the remaining shared haplotypes were found in two strains each. Fourty-six haplotypes were found only once each (Table 1). Analysis of linkage disequilibrium among those loci that showed a relatively high (≥1.20) number of effective alleles revealed 10 pairs of loci that were in significant ($P < 0.05$)

linkage disequilibrium out of a possible 66 pairs. The pairs of loci that were in disequilibrium were AG190 and AC364, AG190 and AC355, AG190 and AC310, AC447 and AC326, AC447 and AC100, AC364 and AC355, AC364 and AC310, AC355 and AC310, AC326 and AC100, and AC310 and AC100.

Accession numbers for ITS sequences are given in Table 1. Using the software within ClustalX, the sequences were aligned, and the output exported to PHYLIP. A dendrogram was constructed using maximum-likelihood. There was no strongly supported structure within the dendrogram, and only the sequences from *P. teres* were clearly separated from the sequences from the rest of the strains belonging to *P. tritici-repentis* and *P. bromi.* Therefore, the dendrogram was not included as a figure. The primary differences among sequences were the presence and absence of small deletions or additions.

DISCUSSION

We used AFLP to determine the level of diversity among these strains because it has been shown to accurately define gene loci (18). The level of diversity revealed by this technique in these strains of *P. tritici-repentis* is reasonably high. The number of effective alleles per locus (9) varies between 1 (monomorphic) and 1.7 (essentially dimorphic), with a large number of strains in our sample carrying unique alleles. This is also reflected in the contributions of the various loci to genetic diversity reflected by Nei's index (27) (Table 2). With a sample size of 97, we could expect to miss some low-frequency alleles altogether. We found four monomorphic (band always present) AFLP loci out of 50 loci scored for *P. tritici-repentis*. Five further loci were defined for either the *P. teres* or *P. bromi* strains used, but there was no corre-

TABLE 1. *Pyrenophora tritici-repentis*, *P. teres*, and *P. bromi* isolates identifying race, amplified fragment length polymorphism (AFLP) haplotype, host, date and location of collection, and GenBank accession number

Isolate	Race[a]	AFLP type[b]	Host from which isolate was collected	Date of collection	Location of collection	GenBank ITS accession number
P. tritici-repentis						
86-124	2	a	Wheat	1980s	Canada	AY739849
SD 97038	1	a	Wheat	1997	South Dakota	AY739782
AKARK B10	1	a	Wheat	2002	Arkansas	AY739792
SDD2-5	1	a	Durum	1999	South Dakota	AY739835
CZ021	3	a	Wheat	2000	Czech Republic	AY739837
CZ012	1	a	Wheat	2000	Czech Republic	AY739838
OK11	1	a	Wheat	1982	Oklahoma	AY739839
K2-2	1	a	Wheat	1980s	Kansas	AY739840
OK98-1-2	1	a	Wheat	1998	Oklahoma	AY739842
PYD7	1	a	Wheat	1969	North Dakota	AY739843
CZ60	1	a	Wheat	2000	Czech Republic	AY739844
CZ1	1	a	Wheat	2000	Czech Republic	AY739845
AK CR B1	1	a	Wheat	2002	Arkansas	AY739860
AK Jac A1	1	a	Wheat	2002	Arkansas	AY739847
ARD-3	1	a	Wheat	1998	Argentina	AY739848
SD97017	1	a	Wheat	1997	South Dakota	AY739850
K3-1	1	a	Wheat	1980s	Kansas	AY739858
ASCI	1	b	Wheat	1980s	Canada	AY739861
SD 4-2	1	b	Smooth brome grass	1998	South Dakota	AY739790
98D-4-16	1	b	Durum	1998	North Dakota	AY739859
98D-5-24	1	b	Durum	1998	North Dakota	AY739801
Ptr 92 119-2-3	1	b	Wheat	1992	North Dakota	AY739803
Ptr 1 1995	1	b	Wheat	1995	North Dakota	AY739806
W1	1	b	Durum	1998	North Dakota	AY739830
AK CR A1	1	b	Wheat	2002	Arkansas	AY739831
1/L1	1	b	Wheat	2002	North Dakota	AY739833
98D 1-1	1	c	Durum	1998	North Dakota	AY739797
CZ033	1	c	Wheat	2000	Czech Republic	AY739841
98D25-3	1	c	Durum	1998	North Dakota	AY739851
98-37-1	1	c	Wheat	1998	North Dakota	AY739852
DW5	5	d	Durum	1998	North Dakota	AY739784
AKPRA A1	1	d	Wheat	2002	Arkansas	AY739781
98-33-28	1	d	Wheat	1998	North Dakota	AY739796
BR25	ND8[c]	e	Wheat	1999	Brazil	AY739776
UR3	2	e	Wheat	1998	Uruguay	AY739786
AK lon A5	1	f	Wheat	2002	Arkansas	AY739787
AKPRA B1	1	f	Wheat	2002	Arkansas	AY739826
BR11	ND8[c]	g	Wheat	1999	Brazil	AY739777
AK cros A1	1	g	Wheat	2002	Arkansas	AY739788
Akjac A5	1	h	Wheat	2002	Arkansas	AY739791
OXEN-1 Ptr	1	h	Wheat	2001	North Dakota	AY739829
ARD 1	ND7[c]	i	Wheat	1998	Argentina	AY739794
98D-3-16	1	i	Durum	1998	North Dakota	AY739795
MD122	4	j	Wild barley	1997	North Dakota	AY739809
MD155	4	j	Smooth brome grass	1997	North Dakota	AY739810
AK Lon A1	1	k	Wheat	2002	Arkansas	AY739827
TSR9-RB11-1	1	k	Wheat	1988	North Dakota	AY739855
SY3-122	4	l	Intermediate wheat grass	1997	North Dakota	AY739819

(*Continued on next page*)

[a] Races 1, 2, 3, 4, and 5 are based on the classification of Lamari et al. (21) and races ND7 and ND8 are based on Meinhardt et al. (26) and Ali and Francl (2), respectively.

[b] AFLP haplotypes are groups of isolates having identical AFLP marker patterns. Absence of a letter entry indicates a unique haplotype for that isolate.

[c] Published race designations (2,25) not corresponding to the accepted race classification proposed by Lamari et al. (21).

sponding band produced from DNA from any of the *P. tritici-repentis* strains tested. Nevertheless, we find that the majority of the strains appear to belong to a single recombining population, as determined by gametic linkage equilibrium. Where there is linkage disequilibrium, this is probably due to relatively low sample sizes and the presence of a few strains that are atypical for the species. If one were to eliminate five strains (usda10a, wb1, wb4, wb5, and 93-9) from the sample, most of the linkage disequilibria would also be eliminated. These strains with linkage disequilibrium may well belong with the other strains in a recombining population, but the sample size is insufficient at this time to give a definitive answer. It is important to note that these strains did not come from geographically isolated regions, but were coincident in origin with the majority of the strains in the sample. Curiously, all of these strains were isolated from non-crop hosts (wild barley or wild rye). This is in contrast to the strains with diverse geographic origins, which were not differentiated from each other genetically.

The level of linkage equilibrium in the sample is surprising given that the sampled strains were not collected in a systematic manner and that *P. tritici-repentis* is homothallic. *A priori* we expected large interpopulational effects, and the effects of selfing, either of which could cause significant deviation from linkage equilibrium. That no such effects were found is further evidence that the strains studied were drawn from one large, stable, interbreeding population. This includes the strains isolated in the Czech Republic, which all had one of the more common haplotypes as did all but one of the strains isolated in South America. The Czech strains could have been introduced from North America, but it is likely that they are not of a single clone as four of the strains share the most common North American haplotype, and the fifth has the third most common North American haplotype.

TABLE 1. (*Continued from preceding page*)

Isolate	Race[a]	AFLP type[b]	Host from which isolate was collected	Date of collection	Location of collection	GenBank ITS accession number
NG18-8	4	l	Intermediate wheat grass	1997	North Dakota	AY739820
Akcros A5	1	m	Wheat	2002	Arkansas	AY739832
OK6	1	m	Wheat	1982	Oklahoma	AY739857
Pti 2	1		Wheat	1973	South Dakota	AY739768
331-9	3		Durum	1980s	Canada	AY739836
93-3	4		Winter barley	1998	North Dakota	AY739824
K1-3	1		Wheat	1980s	Kansas	AY739766
OK12	1		Wheat	1980s	Oklahoma	AY739767
OK 10	1		Wheat	1991	Oklahoma	AY739769
SD 97039	1		Wheat	1997	South Dakota	AY739770
SD Lake	1		Wheat	1997	South Dakota	AY739771
BR1	1		Wheat	1999	Brazil	AY739772
7862	1		Wheat	1978	Montana	AY739773
OK 9	1		Wheat	1983	Texas	AY739774
ARB 1	2		Wheat	1998	Argentina	AY739775
SD Ptr 002	1		Wheat	1997	South Dakota	AY739778
SD97007	1		Wheat	1997	South Dakota	AY739779
88-1	4		Wheat	1980s	Canada	AY739780
AkCros B1	1		Wheat	2002	Arkansas	AY739783
SD97035	1		Wheat	1997	South Dakota	AY739785
Akcr B10	1		Wheat	2002	Arkansas	AY739789
AK Jac B10	1		Wheat	2002	Arkansas	AY739793
98-9-3	1		Wheat	1998	North Dakota	AY739798
98D-1-20	1		Durum	1998	North Dakota	AY739799
98D-2-27	1		Durum	1998	North Dakota	AY739800
Ptr 4B	1		Wheat	1995	North Dakota	AY739802
Ptr 92 148-1-1	1		Wheat	1992	North Dakota	AY739804
98 D-41-1	1		Durum	1998	North Dakota	AY739805
7	4		Basin wild rye	1997	North Dakota	AY739807
22	4		Smooth brome grass	1997	North Dakota	AY739808
NG152	4		Alti wild rye	1997	North Dakota	AY739811
USDA20	4		Needle and thread grass	1997	North Dakota	AY739812
USDA2A	4		Intermediate wheat grass	1997	North Dakota	AY739813
WB5	4		Wild barley	1997	North Dakota	AY739863
MD 164	4		Smooth brome	1997	North Dakota	AY739814
MD112	4		Wild barley	1997	North Dakota	AY739815
SY3-100	4		Intermediate wheat grass	1997	North Dakota	AY739816
NG133	4		Green needlegrass	1997	North Dakota	AY739817
MD 175	4		Smooth brome grass	1997	North Dakota	AY739818
USDA10A	4		Alti wild rye	1997	North Dakota	AY739821
93-9	4		Wild barley	1998	North Dakota	AY739823
WB4	4		Wild barley	1997	North Dakota	AY739862
Ptr 2000-5	1		Wheat	2000	North Dakota	AY739825
WB1	4		Wild barley	1998	North Dakota	AY739828
Marcia9	1		Wheat	2002	North Dakota	AY739834
98-30-1	1		Wheat	1998	North Dakota	AY739846
OK98-2-1	1		Wheat	1998	Oklahoma	AY739853
TSR10-2	1		Wheat	1988	North Dakota	AY739854
OK1	1		Wheat	1982	Oklahoma	AY739856
P. bromi						
SM18A	N/A		Smooth brome	1997	Minnesota	AY739764
P. teres						
ND89-19	N/A		Barley	1989	North Dakota	AY739765

The presence of a large number of haplotypes in the population is not at odds with an interpretation of outcrossing, as the frequencies of these haplotypes falls within the range expected by multiplication of allelic frequencies of the most polymorphic loci, again confirming linkage equilibrium. While the level of apparent outcrossing of this fungus is sufficient to have produced gametic linkage equilibrium for most of its genes, the level of outcrossing which occurs in natural populations cannot be determined from these data. We note a close similarity between the situation in this species with the species *Gibberella zeae* (40). *G. zeae* is also a homothallic ascomycete, and it too displays a high degree of outcrossing as demonstrated by linkage equilibrium among multiple loci as well as a globally unified population.

There is no obvious connection between AFLP haplotype and pathogenic race. Both race 1 and race 4 are found to be very diverse for haplotype, while there are haplotypes shared between races 1 and 2, 1 and 5, 1 and ND7, 2 and ND8, and 1 and ND8. The suggestion is that the species is very diverse for all these traits and with regular outcrossing one might expect a constant renewal of types within a population. This result agrees with that proposed by Lichter et al. (23), where although there was a substantial genomic difference between pathogenic and nonpathogenic races, it was proposed that due to lack of molecular marker polymorphism the isolates were closely related. In our study, the total population of pathogenic and nonpathogenic races (relative to wheat) appears to be closely related, and there is no natural division between different pathogenic races or between pathogenic and nonpathogenic races. This is reasonable if, as we have shown, the population is outcrossing, and it only takes the transfer of a single toxin gene to alter a race.

In work done by Aung (4), random amplified polymorphism DNA marker analysis was used to show major differentiation between pathogenic and nonpathogenic races and also lesser but significant differentiation within the pathogenic (races 1, 2, 3, 5, and 6) population. Differences between pathogenic races were shown to be between necrosis-inducing (races 1 and 2) and non-necrosis-inducing isolates (races 3, 5, and 6) as well as between race 3 and race 5, both of which induce distinctive forms of chlorosis. No significant differences were found between races 1 and 2. In contrast to Aung (4), our work shows that pathogenic races and nonpathogenic races appear to be intercrossing as a single population in nature. The difference in results between Aung (4) and the present work in relation to the differentiation between pathogenic and nonpathogenic strains could partly be due to the sample size of each race. Our work used 21 nonpathogenic race 4 isolates and 76 pathogenic races, whereas Aung (4) used two nonpathogenic isolates and 51 pathogenic isolates, and a sample size of two may not have been large enough to

TABLE 2. Amplified fragment length polymorphism (AFLP) band loci: frequency and descriptive statistics

AFLP band	AG-599	AG-516	AG-503	AG-498	AG-477	AG-462	AG-437	AG-432
Positive	0.01	…	0.97	0.08	0.96	0.01	0.98	…
Negative	0.99	1.00	0.03	0.92	0.04	0.99	0.02	1.00
ne[a]	1.02	1.00	1.06	1.17	1.08	1.02	1.04	1.00
h[b]	0.02	0.00	0.06	0.15	0.08	0.02	0.04	0.00
AFLP band	AG-411	AG-398	AG-385	AG-360	AG-348	AG-322	AG-315	AG-308
Positive	0.03	0.01	0.01	0.11	0.96	0.95	…	1.00
Negative	0.97	0.99	0.99	0.89	0.04	0.05	1.00	…
ne	1.06	1.02	1.02	1.25	1.08	1.11	1.00	1.00
h	0.06	0.02	0.02	0.20	0.08	0.10	0.00	0.00
AFLP band	AG-283	AG-265	AG-259	AG-253	AG-232	AG-219	AG-214	AG-190
Positive	0.06	0.98	0.01	0.02	1.00	0.03	1.00	0.70
Negative	0.94	0.02	0.99	0.98	…	0.97		0.30
ne	1.13	1.04	1.02	1.04	1.00	1.06	1.00	1.71
h	0.11	0.04	0.02	0.04	0.00	0.06	0.00	0.42
AFLP band	AG-169	AG-164	AG-156	AG-151	AG-138	AC-579	AC-558	AC-527
Positive	0.02	0.02	0.97	0.95	0.16	0.94	0.96	0.02
Negative	0.98	0.98	0.03	0.05	0.84	0.06	0.04	0.98
ne	1.04	1.04	1.06	1.11	1.38	1.13	1.08	1.04
h	0.04	0.04	0.06	0.10	0.27	0.11	0.08	0.04
AFLP band	AC-499	AC-493	AC-480	AC-461	AC-447	AC-428	AC-423	AC-419
Positive	0.91	0.01	…	0.92	0.13	0.05	…	…
Negative	0.09	0.99	1.00	0.08	0.87	0.95	1.00	1.00
ne	1.20	1.02	1.00	1.04	1.30	1.11	1.00	1.00
h	0.17	0.02	0.00	0.15	0.23	0.10	0.00	0.00
AFLP band	AC-388	AC-383	AC-364	AC-355	AC-348	AC-326	AC-310	AC-299
Positive	0.95	0.98	0.91	0.91	0.01	0.19	0.76	0.02
Negative	0.05	0.02	0.09	0.09	0.99	0.81	0.25	0.98
ne	1.11	1.04	1.20	1.20	1.02	1.45	1.59	1.04
h	0.10	0.04	0.17	0.17	0.02	0.31	0.37	0.04
AFLP band	AC-287	AC-276	AC-145	AC-138	AC-118	AC-100	AC-97	
Positive	0.32	0.99	1.00	0.99	0.07	0.62	0.98	
Negative	0.68	0.01	…	0.01	0.93	0.38	0.02	
ne	1.76	1.02	1.00	1.02	1.15	1.88	1.04	
h	0.43	0.02	0.00	0.02	0.13	0.47	0.04	

[a] ne = effective number of alleles (Kimura and Crow [19]).
[b] h = genetic diversity index (27).

identify significant outcrossing within the population. In our study, all but three of our pathogenic races were necrosis-producing isolates and therefore this set of isolates would neither be sufficient to detect differences between necrosis- and nonnecrosis-producing isolates nor between races producing different forms of chlorosis.

In addition, the data presented here do not show clear divisions between different species of the genus *Pyrenophora*. Sequence analysis of the ITS region fails to separate *P. tritici-repentis* from *P. bromi* (although there is some differentiation between *P. tritici-repentis* and *P. teres*). Also, in one case, AFLP similarity is greater between a *P. tritici-repentis* isolate (Ptr 2000-5) and a *P. teres* isolate (ND89-19) than between the same *P. tritici-repentis* isolate and other isolates from the same species. While, in the majority of cases, there is clear differentiation between the species on the AFLP level, the number of shared bands among all three species suggests close genetic affinities.

In a previous study using ITS sequence to investigate the genus *Pyrenophora*, Zhang and Berbee (41) used two isolates of *P. tritici-repentis* and a single isolate each of *P. bromi* and *P. teres*. This work showed that using ITS sequence, *P. tritici-repentis* and *P. bromi* grouped together and *P. teres* was also closely related. Our results are compatible with those of Zhang and Berbee (41), with the added observation that our ITS data do not separate *P. bromi* and *P. tritici-repentis*, but rather we find that the ITS sequences from some *P. tritici-repentis* isolates are more similar to the *P. bromi* ITS sequences (both our own and those previously published) than they are to other *P. tritici-repentis*-derived ITS sequences.

The work presented here has laid the foundation for future studies by providing valuable information on the genetic diversity of the worldwide population of *P. tritici-repentis*. These studies may include a larger more diverse study incorporating more isolates from all continents and wheat growing regions of the world. This study has also revealed that a comparative analysis of other cereal pathogen species of the genus *Pyrenophora* should be undertaken.

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